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Behavioral Effects of THC as a Function of Environment and Prior Drug Experience

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MARTIN, P., W. HODGE, M. ROYAL AND B. JONES. *Behavioral effects of THC as a function of environment and prior drug experience.* PHARMACOL BIOCHEM BEHAV **26**(1) 141–144, 1987.—Holtzman albino rats were divided into 4 groups, and on 5 consecutive days each group was exposed to one of 4 conditions. The drug-adapted group was given delta-9-tetrahydrocannabinol (THC) (0.0, 0.5, 2.5 or 5.0 mg/kg PO) in their home cages, while the environment-adapted group was given vehicle and placed for one hr in the chamber where they were later tested. The naive group was given vehicle in their home cages and the drug + environment adapted group was given THC and placed in the test chamber. One week later, all rats were given either 0.0, 0.5, 2.5, or 5.0 mg/kg THC and placed in the test chamber where standing, sitting, and behavioral activity were measured. The results showed that the behavioral effects of THC are a function of environmental familiarity in rats who are drug naive but not in rats given prior exposure to THC.

Δ^9-Tetrahydrocannabinol Rats Behavior Environment Adaptation Drug adaptation

DURING the past thirty years, the area of behavioral pharmacology has shown that the effects which one dose of a drug has upon a particular response pattern depends upon a wide variety of environmental factors. For example, a single dose of a drug can increase or decrease response rate depending upon the current schedule of reinforcements [3], the type of reinforcer used to maintain the behavior [5], the subject's prior experience with the drug [9], prior experience with other schedules of reinforcement [2] and prior experience with other reinforcing or punishing events [1,2].

While these studies demonstrate the importance of the organism's history and current environment in understanding the effect of drugs on learned behaviors, experiments which focus on the neurochemical mechanisms of drugs primarily have used spontaneously occurring, unlearned behaviors such as aggression or open field activities as the behavioral measure. In these experiments, the previous history and current environment of the organism usually (but not always) are recognized as factors to be controlled, in the sense that, for a given experiment, care is taken to insure that all animals are experimentally naive or have similar experimental histories, and are housed in a similar way. However, the events which occur in the life of an organism seldom are examined as variables which interact with the drug that is being studied. The results of the experiments in which these interactions have been studied though, suggest that the interactions are potent ones.

One example of this type of interaction is found in rabbits who have been given (−) delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana. Both the behavioral and the electroencephalographic (EEG) effects of THC are dependent upon how familiar animals are with the observation chamber in which they are tested [4]. More specifically, animals who had been adapted to the chamber showed dose-dependent decreases in activity, with concomitant increases in sprawling and cortical voltage output. However, rabbits who were not adapted to the chamber exhibited both behavioral and EEG activation when given an equivalent dose of THC. Furthermore, when THC was administered to adapted and non-adapted rabbits over a twelve day period, activity levels of the adapted rabbits increased as tolerance developed, while the activity level of the non-adapted rabbits did not [7]. In both of these experiments, however, only one dose of THC was used. Consequently, the first purpose of the present experiment was to examine more closely the interaction of both environmental familiarity and drug familiarity with a range of doses of THC. A second purpose of the experiment was to extend these findings to another species, *Rattus norvegicus*.

METHOD

Subjects

Subjects in this experiment were 160 male Holtzman albino rats, who ranged in weight from 250–300 grams. They were housed in individual cages under controlled lighting (12 hr light-dark) and given free access to food and water except during the pretreatment and treatment sessions. During those times they were maintained on a 23 hr water deprivation schedule.

Drugs

Trans (−) delta-9-tetrahydrocannabinol was provided by the National Institute on Drug Abuse. It was prepared at a concentration of 2.5 mg/ml in a vehicle of 1.0% Tween-80, 9.0% sesame oil, 60.0% Coco Lopez and 30.0% water.

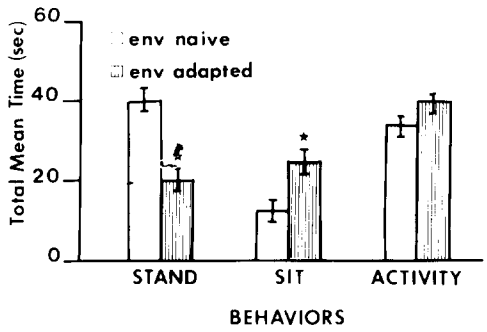

FIG. 1. Environment Adaptation Main Effect. The total mean time spent engaged in each behavior is shown for rats who were naive or adapted to the open field. Treatment groups are shown on the abscissa while the total mean time is shown on the ordinate.

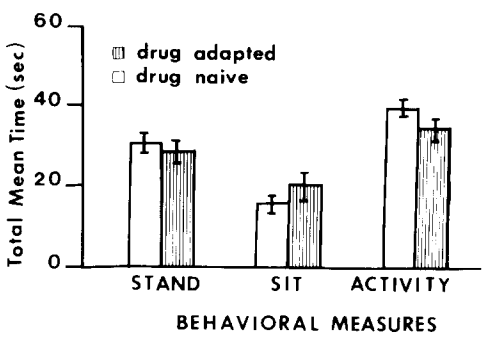

FIG. 2. Drug Adaptation Main Effect. The total mean time spent engaged in each behavior is shown for rats who were naive or preexposed to THC. Treatment groups are shown on the abscissa while the total mean time engaged in each behavior is shown on the ordinate.

Apparatus

The apparatus consisted of a 1.14×1.14 m square open field, with walls 23 cm high. The open field was painted white, and was divided into 16 squares by black grid lines painted across the floor. Each square was 28.5 cm in size. An Apple II+ microcomputer was used by an observer to record the frequency and duration of six behaviors during the time that the animal was in the open field.

Dependent Variables Measured

Frequency and duration of the following behaviors were measured: (a) Standing—weight of animal supported on legs. (b) Sitting—weight of animal distributed along the ventral body surface. (c) Activity—activity was scored as the total time engaged in the following behaviors: (1) Locomotion—number of grid crossings defined as movement of 2 limbs from one section to another. (2) Grooming—licking or scratching directed towards the animal's body. (3) Rearing—standing up with front paws off the floor of the chamber. (4) Exploring—sniffing at the chamber or object in the chamber with extended head and vibrissae movement.

Procedure

Naive rats. Four groups of animals, with 10 animals per group, were pretreated with an oral dose of vehicle daily for 5 days. One week later, animals were given a single dose of vehicle, 0.5, 2.5, or 5.0 mg/kg of THC. Thirty min later they were placed in an open field and the frequency and duration of each of the six behaviors defined above were recorded for 30 sec once every 10 min over a 30 min test session. The behaviors were defined so as to be mutually exclusive, and more than one behavior could be recorded during each observation period.

In rodents, the plasma concentration of THC is low following oral administration of 5.0 mg/kg of THC and it remains fairly constant from 30 min to approximately 4 hr after administration [6]. Consequently, a 30 min period between drug administration and the initiation of testing was used in this experiment. All testing was done using a double-blind procedure in which one experimenter randomly assigned animals to groups and administered the experimental treatment, while two others, who were blind to the treatment conditions, did the behavioral observations. The two observers were trained before the experiment began, and inter-

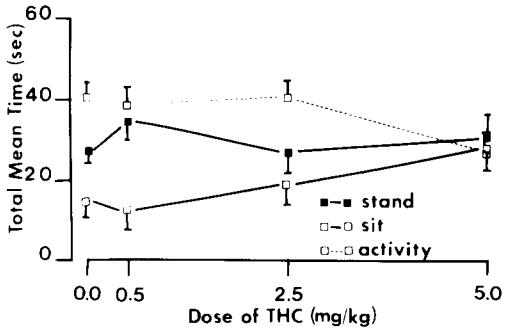

FIG. 3. Dose Main Effect. The total mean time spent engaged in each behavior is shown for rats receiving 0.0, 0.5, 2.5 or 5.0 mg/kg of THC, PO. Treatment groups are shown on the abscissa while the total mean time engaged in each behavior is shown on the ordinate.

observer reliability was evaluated twice, once prior to the experiment and once during the experiment. During each evaluation, the two observers independently and simultaneously recorded the behaviors of approximately 4 rats tested successively. After each evaluation session, Pearson product-moment correlation coefficients were calculated for the data from the two observers, and the correlation coefficients were found to be .90 for the first evaluation session and .92 for the second evaluation session.

Drug adapted rats. Four groups of animals (n=10) were pretreated with either vehicle, 0.5, 2.5 or 5.0 mg/kg of THC in their home cages for 5 days. One week later they were given a single administration of the same dose (either vehicle, 0.5, 2.5 or 5.0 mg/kg) of THC, and thirty min later they were placed in the open field and tested as described above.

Environment adapted rats. Forty rats (n=10) were pretreated with vehicle and then placed in the open field one hour daily for 5 days. One week later, they were given a single administration of the same dose (either vehicle, 0.5, 2.5, or 5.0 mg/kg) of the THC and tested as described above.

Environment plus drug adapted rats. Forty rats (n=10) were pretreated with either vehicle, 0.5, 2.5, or 5.0 mg/kg of THC and then placed in the open field one hour daily for 5 days. One week later they were given a single dose (either vehicle, 0.5, 2.5 or 5.0 mg/kg) of THC and tested as described above.

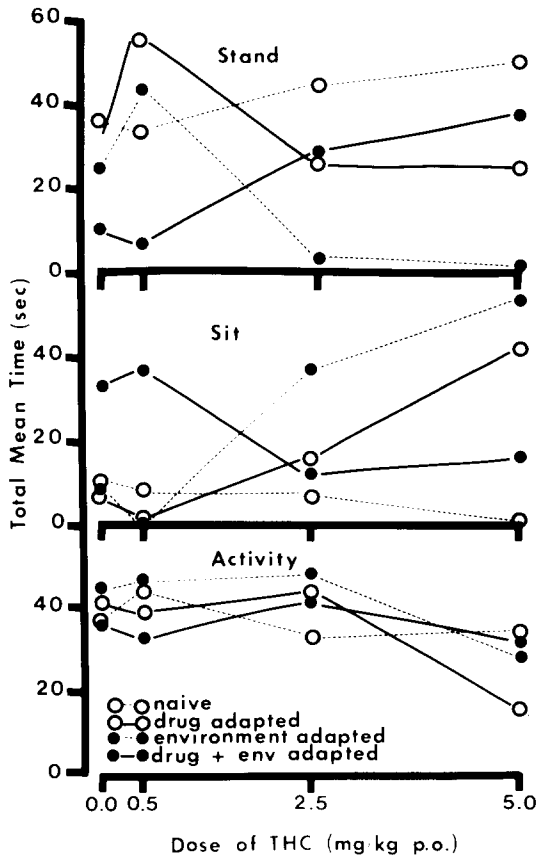

FIG. 4. Environment Adaptation × Drug Adaptation × Dose Interaction. The total mean time spent engaged in each behavior as a function of dose is shown for each of the four treatment groups. Doses of THC are shown on the abscissa while the total mean time engaged in each behavior is shown on the ordinate.

RESULTS

The experiment was designed as a 2 (environmental adaptation) × 2 (drug adaptation) × 4 (dose) factorial. Univariate random groups analyses of variance (ANOVA) were used to analyze the data for each dependent variable, and Newman-Keuls post-hoc tests were used to make specific comparisons of individual groups.

Main Effects

As can be seen in Fig. 1, animals who were adapted to the open field prior to testing spent significantly less time standing, $F(1,444)=31.8$, $p<0.00001$, and more time sitting, $F(1,144)=12.85$, $p<0.0001$, than did animals who were unfamiliar with the test chamber. However, these two groups did not differ in overall activity level.

The drug adaptation main effects, shown in Fig. 2, did not reveal any overall differences between drug adapted and drug naive rats on any of the behavioral measures.

Finally, the dose main effects, shown in Fig. 3, indicated a dose-dependent increase in sitting, $F(3,144)=3.42$, $p<0.05$, and decrease in activity, $F(3,144)=3.55$, $p<0.05$, but no dose-dependent change in standing. Newman-Keuls post-hoc tests showed that animals who received 5.0 mg/kg of THC spend significantly more time sitting and less time engaged in activity than did animals receiving lower doses ($p<0.05$ for each comparison).

Interactions

There were no significant two-way interactions. However, there were significant environmental adaptation × drug adaptation × dose interactions for standing, $F(3,144)=15.996$, $p<0.00001$, and sitting, $F(3,144)=15.04$, $p<0.00001$, but not for activity. As can be seen in Fig. 4, there was no dose-dependent change in the amount of time naive animals spent standing. In contrast, the animals who were adapted to the environment showed a very strong reduction in standing as a function of dose ($p<0.05$ for each comparison). Animals who had received prior exposure to THC showed an increase in standing following the lowest dose of THC ($p<0.05$), but returned to vehicle level following the two higher doses of THC. Animals adapted to both the drug and the open field spent less time standing following vehicle than did the other three groups ($p<0.05$ for each comparison), and showed a dose-dependent increase in standing as the dose of THC increased ($p<0.05$ for each comparison).

As can also be seen in Fig. 4, naive animals spent little time sitting, and again, showed no dose-dependent changes in this behavior. Environment adapted animals exhibited a high dose-dependent increase in sitting ($p<0.05$ for each comparison), as did animals who had received prior exposure to THC. However, animals adapted to both, like naive animals, showed no significant change in sitting.

DISCUSSION

These results show first, and most obviously, that when animals have never been exposed to THC, the behavioral effects of THC are a function of the animal's familiarity with the environment in which he is tested. That is, rats who are in a familiar environment exhibit dose-dependent decreases in activity level and standing, and a concomitant increase in sitting. Rats in an unfamiliar environment, however, exhibit dose-dependent decreases in activity level and sitting, with a concomitant increase in standing. These results are very similar to those found in New Zealand White rabbits [4,7], and extends these findings to another species, *Rattus norvegicus*.

The results of the present experiment further extends those of others [4,7] by showing that when animals have had prior experience with THC, familiarity with the environment does not play as great a role in determining how the animals behave when given THC. Although the behavioral patterns of the two groups differed as a function of environmental familiarity when they were given vehicle control and the lower dose of THC, the amount of time spent engaged in sitting, standing and activity did not differ for these two groups following the two higher doses of THC. Furthermore, both groups of THC-exposed animals spent more time standing than did drug-naive animals who were given THC for the first time in a familiar environment. These results are consistent with other studies showing that both rabbits [7] and rats [10] show increased standing, vigilance, and hesitancy in moving after subacute and chronic THC administration. In this respect, the present data are especially interesting, since the animals in this experiment received THC only five times and then went through a drug free period of at least one week before they were tested. Apparently, the behavioral stimulation that occurs with repeated exposure to THC develops

quickly and persists for a long period of time after THC exposure.

The finding that animals in all treatment conditions showed a dose-dependent decrease in activity level is consistent with other experiments showing a biphasic response to THC, consisting of CNS-depression for several days, followed by CNS stimulation [8]. Rats generally show hyperactivity in an open field when THC is administered for more than 10 days [8, 10, 11] but are hypoactive when THC is administered for shorter periods of time [8]. However, these results are inconsistent with one study showing that rabbits given a single dose of THC and placed in an unfamiliar environment become very active compared to placebo controls. This could be due to species differences [7].

In conclusion, these results show that the behavioral effects of THC are a function of both the animal's familiarity with the test chamber and his prior experience with the drug Since the behavioral measures which were used in this particular experiment also are used frequently in experiments which focus on mechanisms of action, these results further suggest that any mechanisms which are identified might be specific to a particular set of environmental conditions. Consequently, these environmental conditions should be systematically examined in mechanism studies, as variables which modulate the actions of drugs.

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